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# Create A Case

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| ☑ | USPT | L2 and flexible linker | YES | ADJ | ASSIGNEE | L3 |

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L2: Entry 1 of 39 File: USPT Mar 18, 2003

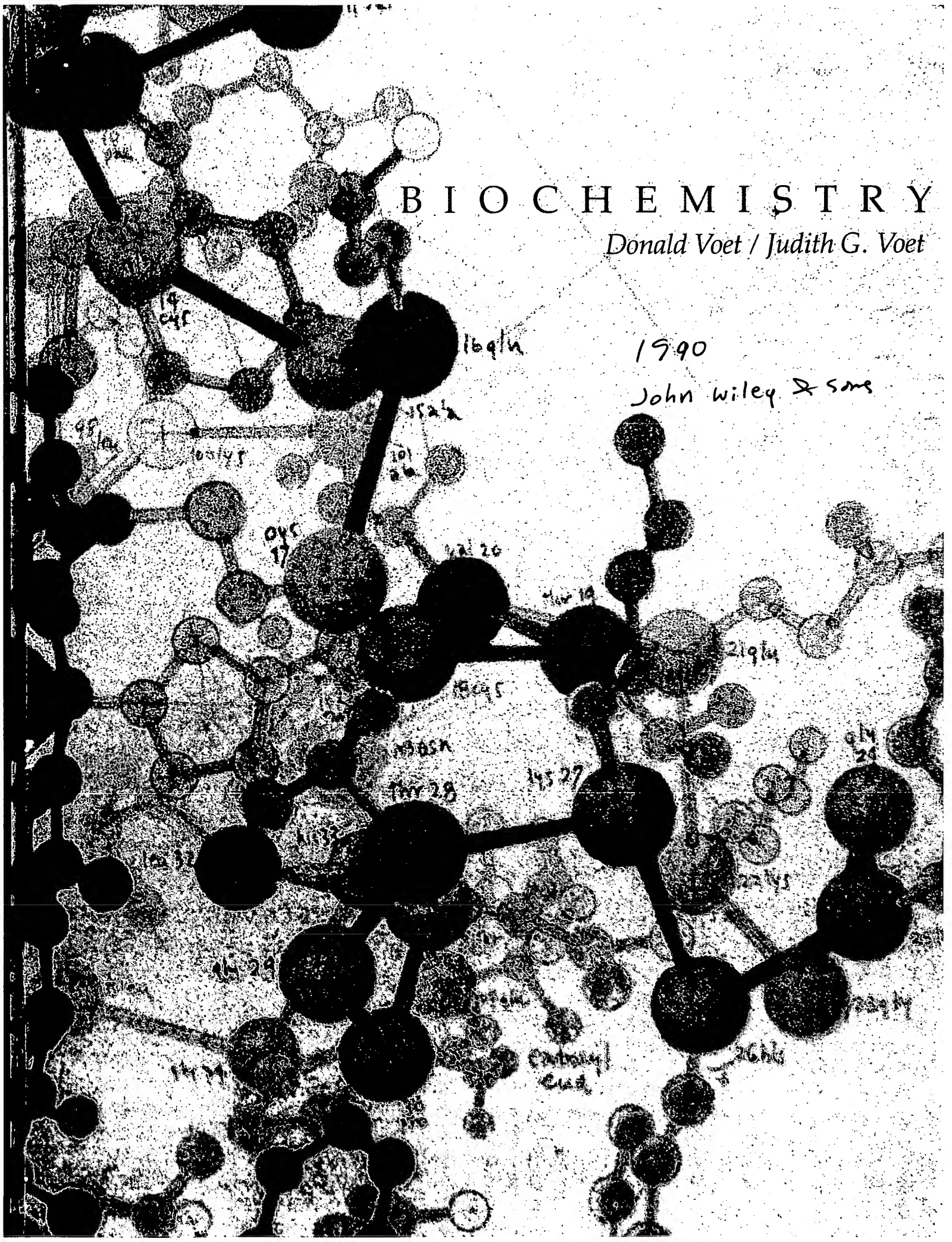
DOCUMENT-IDENTIFIER: US 6534301 B2
TITLE: 16835, a novel human phospholipase C family member and uses thereof

Detailed Description Text (128):
The term "antigen-binding fragment" of an antibody (or simply "antibody portion," or "fragment"), as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to the antigen, e.g., 16835 polypeptide or fragment thereof. Examples of antigen-binding fragments of the anti-16835 antibody include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab').sub.2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

Detailed Description Text (145):
The anti-16835 antibody can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Coicher, D. et al. (1999) Ann NY Acad Sci 880:263-80; and Reiter, Y. (1996) Clin Cancer Res 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target 16835 protein.

# BIOCHEMISTRY

*Donald Voet / Judith G. Voet*



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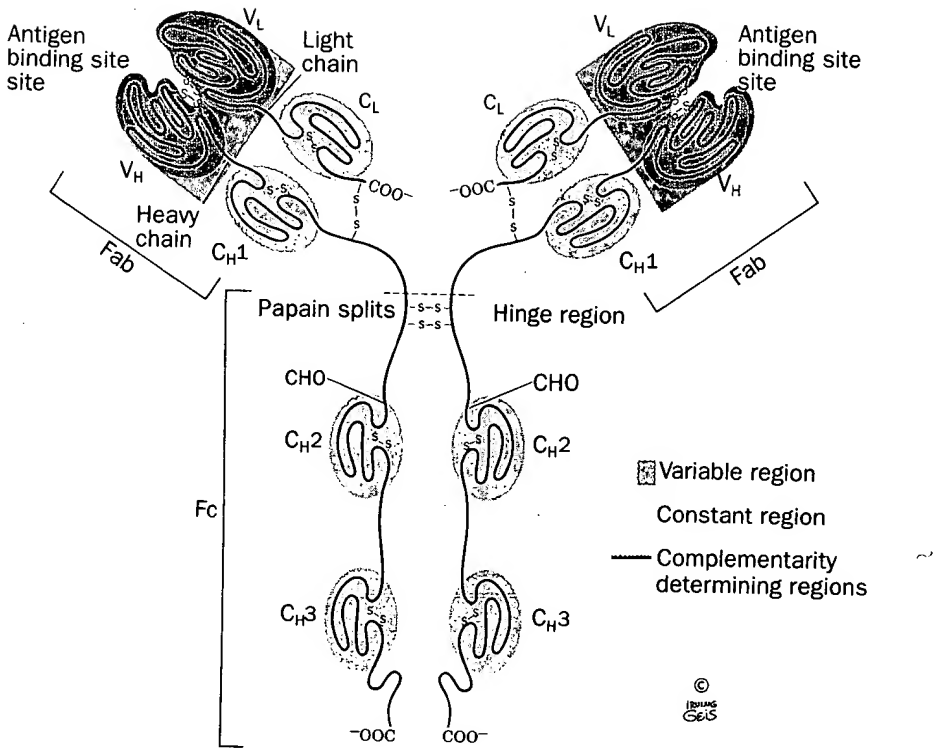


**Figure 34-15**
A diagram of the human IgG molecule. Each light (L) chain consists of two homologous units, $V_L$ and $C_L$, where V and C indicate the polypeptide chain's variable and constant regions. Each heavy (H) chain is composed of four such units, $V_H$, $C_H1$, $C_H2$, and $C_H3$. Treatment of IgG by the proteolytic enzyme papain results in the cleavage of this immunoglobulin molecule in its "hinge" region yielding two Fab fragments and one Fc fragment. CHO represents carbohydrate chains. [Drawing copyrighted © by Irving Geis.]

*ity for self-antigens are selected for further propagation.* Indeed, only a small fraction of the lymphocytes that are processed by the thymus ever leave that organ.

Occasionally, the immune system loses tolerance to some of its self-antigens resulting in an **autoimmune disease.** For example, **myasthenia gravis,** an autoimmune disease in which individuals make antibodies against the **acetylcholine receptors** of their own skeletal muscles (acetylcholine is a neurotransmitter that triggers muscle contraction; Section 34-4C), results in a progressive and often fatal muscular weakness. Similarly, individuals with **systemic lupus erythematosis,** an often fatal inflammatory disease, produce antibodies against many of their own cellular components including certain ribonuclear proteins (Section 29-4A). Other common autoimmune diseases are **rheumatoid arthritis, insulin-dependent diabetes mellitus** (Section 25-3B), and **multiple sclerosis.**

## B. Antibody Structures

The immunoglobulins form a related but yet enormously diverse group of proteins. In this section, we consider the structures of these essential molecules. How their diversity is generated is the subject of the following section.

### There Are Five Classes of Immunoglobulins

Most immunoglobulins, and the basic building blocks of all of them, consist, as Gerald Edelman and Rodney Porter showed, of four subunits: two identical ~23-kD **light chains (L)** and two identical 53- to 75-kD **heavy chains (H).** These subunits associate via disulfide bonds as well as by noncovalent interactions to form, as electron micrographs indicate, a Y-shaped symmetric dimer, $(L-H)_2$ (Fig. 34-15). Immunoglobulins are glycoproteins; each heavy chain has an *N*-linked oligosaccharide.

*Humans have five classes of secreted immunoglobulins, designated IgA (for **immunoglobulin A**), IgD, IgE, IgG, and IgM which differ in their corresponding types of heavy chains designated $\alpha$, $\delta$, $\varepsilon$, $\gamma$, and $\mu$, respectively (Table 34-2). There are also two types of light chain, $\kappa$ and $\lambda$, but these occur in immunoglobulins of all classes.* IgD, IgE, and IgG exist only as $(L-H)_2$ dimers. IgM, however, consists of pentamers of its respective dimers and IgA occurs as monomers, dimers, and trimers of its corresponding dimers (Fig. 34-16). The dimeric units of these multimers are linked by disulfide bonds to each other and to an ~20-kD protein termed the **joining chain (J).** IgM also occurs in a *B* cell-displayed monomeric membrane-bound form. It is antigen binding by this latter form of IgM that triggers the humoral immune response.

# Antibody Engineering

## A Practical Guide

Carl A. K. Borrebaeck
Editor

1992

W. H. Freeman and Company

New York

# CHAPTER 1

# Antibody Structure and Structural Predictions Useful in Guiding Antibody Engineering

*Arthur M. Lesk, Anna Tramontano*

Structural analysis of antibodies, based on the few known structures and the many known sequences, should provide answers to several important questions about the functional properties of antibodies and guide attempts to engineer changes in them. The questions include

- What are the common features of antibody structures that form the basis of the properties shared by different immunoglobulins?
- Where in the structure does the antigen-binding site reside? As is well known, Kabat and his coworkers recognized certain regions of the sequences as hypervariable and suggested—correctly and presciently—their involvement in antigen binding,[1, 2] a conclusion that was later confirmed by analysis of crystal structures of antibodies.
- What is the nature of the antigen-antibody interaction?
- How is the diversity in antigen-binding specificity achieved, in terms of changes in three-dimensional structure?
- How, during the maturation of the antibody response, are antigen-binding sites progressively "tuned" in affinity?

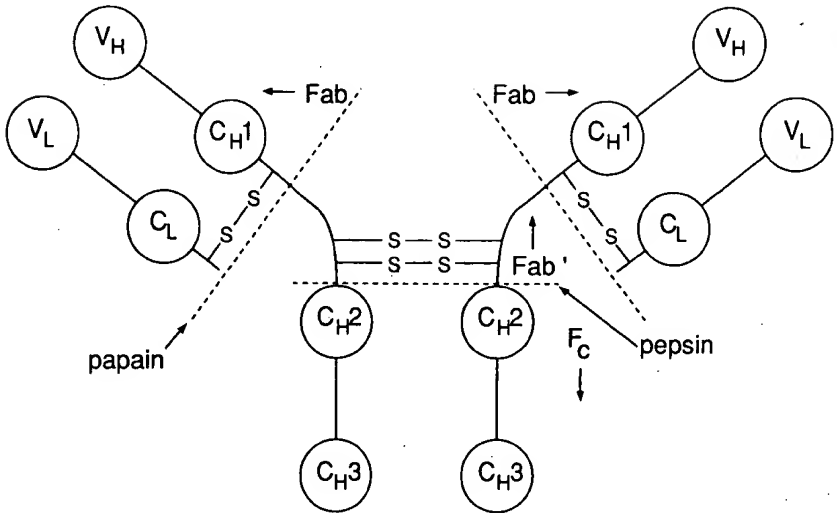


Figure 1-1. (A) A schematic diagram of the structure of an IgG, showing the distribution of domains in the heavy and light chains, and the interchain domain bridges.

variable and one constant domain, denoted $V_L$ and $C_L$; and two identical heavy chains each containing one variable and three constant domains, denoted $V_H$, $C_H1$, $C_H2$, and $C_H3$. Figure 1-1A also shows the disulfide bridges linking the chains together. In addition, immunoglobulins contain carbohydrate moieties that are not indicated in this figure.

Limited proteolytic cleavage with enzymes (or cyanogen bromide) produces well-defined fragments of the molecule, containing different combinations of domains. Figure 1-1B defines these fragments.

## CRYSTAL STRUCTURE DETERMINATIONS

X-ray crystal structure analyses of immunoglobulins have been carried out on intact antibodies, Bence Jones proteins consisting of light chain dimers, Fc fragments, and (most commonly) Fab or Fab′ fragments (Table 1-1). Crystal structures of Fab fragments include some unligated molecules and others that contain bound antigens. In some cases, the structure of an antibody fragment is known in both the unligated state and with antigen bound; these cases are important because they reveal the conformational changes that occur upon ligation of antigen, a subject of considerable interest.

Table 1-1 lists the crystal structures deposited in the protein data bank.[6-19] The early work was done on myeloma proteins. The availability of monoclo-